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Research Article

ANTIBACTERIAL ACTIVITY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY STUDIES OF ALGERIAN *ATRIPLEX HALIMUS* L.

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ABSTRACT

Objective: The objective of the study was to find out the antibacterial efficacy and identify the main constituents of the essential oil of *Atriplex halimus* from southwest of Algeria.

Methods: The essential oil from the aerial parts of the endemic plant *A. halimus* (saltbush in English, qataf in Arabic) collected from the region of Sahara southwest of Algeria was isolated by hydrodistillation and analyzed by gas chromatography–mass spectrometry. Antibacterial potency of essential oil from this plant has been tested against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Bacillus cereus* (ATCC11778) by disk diffusion assay.

Results: The essential oil revealed the presence of 14 components, the dominant compounds arecadina-1(10), 4-diene (10.69%), germacrene D (9.79%), octane (9.37%), pelargonaldehyde (9.06%), 3-Furancarboxaldehyde (6.87%), β-pinene (2.6%), camphene (2.59%), and myrcene (2.10%). The essential oil exhibits very effective antimicrobial activity using disk diffusion assay method with minimum inhibitory concentration ranging from 0.82 µl/ml to 2.4525 µl/ml.

Conclusions: This result showed that this native plant may be a good candidate for further biological and pharmacological investigations.

Keywords: Essential oil, *Atriplex halimus*, Antimicrobial activity, Gas chromatography–mass spectrometry analysis.

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INTRODUCTION

Atriplex species of the family Amaranthaceae (formerly Chenopodiaceae) are among the few salt-tolerant plants which collected the salt lands in bladder cells situated on leaf surfaces and subsequently excreted by the bursting of these cells [1,2]. *Atriplex halimus* is an evergreen shrub which can grow 2 m high and 3 m diameter at a medium rate. It is a monoecious plant that is pollinated by wind and produces a staminate flower [3]. It is widely distributed in Europe and Northern Africa, including the Sahara in Morocco and Algeria [4] (Fig. 1).

A. halimus is used as fodder reserves and as a supplementary forage resource in arid and semi-arid countries [5]. The ensiling *A. halimus* as a browse forage showed comparable results to polyethylene glycol (PEG) supplementation and might be easier and might lower feeding cost than daily PEG supplementation [6].

A. halimus plants accumulate large amounts of Cd in their tissues (predominance in roots), suggesting the possibility of their use in decontaminating saline soils polluted by Cd [7].

In Algeria and in particular, the Sahara part, the plant is used by Bedouin for feeding their sheep and goats [4], and in popular folk remedy, it is used to treat diabetes, stomach pains, chest ailments, muscular pain, and intestinal worms and to regulate gallbladder excretions.

Regarding the chemical composition of this species, and to the best of our knowledge, few chemical compositions have been reported in the literature [8,9]. In these papers, authors describe the isolation of some phenolic compounds, including flavonoids. We report herein the chemical composition of the essential oil of *A. halimus* growing in Algeria, and some carried antibacterial activities.

METHODS

Plant material

The aerial parts (leaves and stems) of *A. halimus* were collected in March 2016 from the region of Bechar, Algeria and identified. A voucher specimen was deposited at the Herbarium of the Valorization of Resource and Food Security in Semiarid Areas Laboratory, South West of Algeria, University of Bechar [10,11].

Extraction of essential oil

The dried aerial parts of *A. halimus* (1 kg) were subjected to hydrodistillation for 5 h in 3 times using a Clevenger-type apparatus, according to the method outlined by the European Pharmacopoeia [12]. The essential oil was then separated from the aqueous layer, dried over anhydrous sodium sulfate. The calculated average of essential oil yield is 0.0475%. The essential oil was stored in sealed vials at low temperature (4°C) until (GC– MS) analysis.

GC–MS analysis

The GC–MS analysis was performed using a Hewlett Packard Agilent 6890 GC system coupled with a 5973C MS. HP-5 MS analytical fused silica capillary column (60 m × 0.25 mm × 0.25 µm, Agilent, Santa Clara, CA) was used for chromatographic separations. For both columns, the oven temperature had ramped from 60°C to 250°C (8 min) at 2°C/min isothermal for 10 min. The flow rate of the helium was 0.5 mL/min. The retention indices for all components were determined according to the method using n-alkanes as standard.

Identification of components

Individual peaks were identified by comparing their Kovats index relative to n-alkanes (C8-C29) obtained by a non-polar HP-5MS column provided in the literature running at the same conditions used for the

Table 1: Chemical composition of the essential oil of *Atriplex halimus*

Peak	RT	Name of compound	Formula	Area (%)	RI
1.	4.713	Octane	C_8H_{18}	9.37	80,057,554
2.	5.028	3-Furancarboxaldehyde	$C_5H_4O_2$	6.87	80,964,029
3.	5.628	2-vinyl-5-methylfuran	C_7H_8O	2.16	82,690,648
4.	6.239	1,1,4-Trimethylcyclohexane	C_9H_{18}	3.83	84,448,921
5.	11.366	Camphene	$C_{10}H_{16}$	2.59	95,333,556
6.	12.812	β-Pinene	$C_{10}H_{16}$	2.6	97,745,163
7.	13.612	Myrcene	$C_{10}H_{16}$	2.1	99,079,386
8.	14.881	a-Terpinene	$C_{10}H_{16}$	3.24	10,101,573
9.	21.641	Pelargonaldehyde	$C_9H_{18}O$	9.06	11,058,323
10.	25.456	p-Menthan-3-one, cis-p-	$C_{10}H_{18}O$	2.88	11,590,624
11.	32.845	Thiophene,2-[(methylthio)ethynyl]	$C_{10}H_{16}O$	3.11	12,647,435
12.	36.051	Unknown		8.29	13,119,705
13.	46.991	Germacrene D	$C_{15}H_{24}$	9.79	14,845,492
14.	48.991	Myristicin	$C_{11}H_{12}O_3$	4	15,179,931
15.	49.374	Cadina-1(10),4-diene	$C_{15}H_{24}$	10.69	15,245,626
16.	59.193	Unknown		8.59	16,981,887
17.	66.812	Unknown		2.77	1,745,191
Number of identified compounds				14	
Monoterpene hydrocarbons				23.73	
Oxygenated monoterpenes				32.62	
Sesquiterpene hydrocarbons				15.94	
Others compounds				19.65	
Percentage of identified compound				91.94	

RI: Retention indices relative to C8-C29 *n*-alkanes on the HP-5MS column; RT: Retention time

Table 2: Antimicrobial activity of essential oils from the aerial parts of *Atriplex halimus*

Organisms	Minimum inhibitory concentration (µl/ml)
Escherichia coli	1.64
Staphylococcus aureus	2.4525
Bacillus cereus	0.82

Fig. 1: Photograph of *Atriplex halimus* [4]

essential oils and by comparing their mass spectral fragmentation patterns using NIST08 library spectra database [13].

Antibacterial activity

The disk diffusion method was used to screen for antibacterial activities of the crude extracts against the four bacterial strains, namely, *Staphylococcus aureus* (ATCC 29213, positive control: Penicillin), *Escherichia coli* (ATCC 25922, positive control: Gentamycin sulfate injection), and *Bacillus cereus* (ATCC11778). The Petri dishes were maintained by serial subculturing every month on nutrient agar slants and incubating at 37°C for 18–24 h. The minimum inhibitory concentrations (MICs) of the extracts were determined using a serial microplate dilution assay against each test bacterial species.

RESULTS AND DISCUSSION

Essential oil analysis

The yield of the essential oil (light yellow color) obtained by hydrodistillation of *A. halimus* was 0.0475%, and a total of 17 different volatile and semi-volatile compounds were identified (Table 1), distributed by distinct chemical classes: Oxygenated monoterpenes (32.62%), sesquiterpene hydrocarbons (15.94%), monoterpene hydrocarbons (23.73%), and sulfur compounds (3.11%). Nearly 20% of the oil composition is unknown, which might be due to insufficient information about their RI in literature.

The number of components identified in the essential oil of *A. halimus* is 14 which accounted for 91.94% of the total components. Their retention indices and relative percentages are shown in Table 1. The dominant constituents identified in the sample are cadina-1(10)4-diene (10.69%) [14], germacrene D (9.79%) [15], octane (9.37%) [14], pelargonaldehyde (9.06%) [16], and 3-Furancarboxaldehyde (6.87%) [17]. Other minor constituents of the oil are myristicin (4.00%) [18], 1,1,4-Trimethylcyclohexan (3.83%), α-terpinene (3.24%) [19], Thiophene,2-[(methylthio) ethynyl] (3.11%) [18], p-Menthan-3-one, cis-p- (2.88%) [14], β-Pinene (2.60%) [15], camphene (2.59%) [18], 2-vinyl-5-methylfuran (2.16%) [16], and myrcene (2.10%) [18]. These qualitative and quantitative differences in the chemical composition of essential oils could be attributed to several factors such as geographical location, the climatic effects, harvest season, nature of the soil, age of the plant parts, the state of used plant materials (dried or fresh), the part of the plant used, time of collection, and chemotype [20,21].

Antibacterial activity

The results obtained for antibacterial activity screening of *A. halimus* essential oil are summarized in Table 2. With the broth dilution method, the MIC values for essential oil of aerial parts were in the range of 0.82–2.4525 µl/ml.

The essential oil of *A. halimus* was found to have moderate to high antimicrobial activity. It showed strong inhibition against *B. cereus* and low activity against *S. aureus*. This antimicrobial activity may be due to the chemical composition of the essential oil, which is rich in oxygenated monoterpenes.

CONCLUSIONS

This paper presents an interesting analysis of the chemical composition of the aerial parts essential oil of *A. halimus*. Among the 14 components identified, cadina-1(10)4-diene (10.69%), germacrene D (9.79%), octane (9.37%), and pelargonaldehyde (9.06%) were the main components. The antibacterial activity of *A. halimus* oil displayed a significant effect among the different bacterial strains.

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AUTHORS' CONTRIBUTIONS

The author declares that this work was done by the author named in this article.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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